American Journal of Medical Genetics 68:494–498 (1997)

RAPID PUBLICATION

ASSOCIATION OF A POLYMORPHIC VARIANT OF THE WERNER HELICASE GENE WITH MYOCARDIAL INFARCTION IN A JAPANESE POPULATION

Lin Ye, Tetsuro Miki, Jun Nakura, Junko Oshima, Kouzin Kamino, Hiromi Rakugi, Hiroshi Ikegami, Jitsuo Higaki, Steven D. Edland, George M. Martin, and Toshio Ogihara

Department of Geriatric Medicine, Osaka University Medical School[1], *(L.Y., T.M., J.N., K.K., H.R., H.I., J.H., T.O.) and Department of Pathology (J.O., G.M.M.), Department of Environmental Health and Biostatistics (S.D.E.), University of Washington School of Medicine*

The Werner syndrome (WS) is a rare autosomal recessive progeroid syndrome characterized by the premature onset of multiple age-related disorders, including atherosclerosis, cancer, non-insulin-dependent diabetes mellitus (NIDDM), ocular cataracts and osteoporosis [Epstein et al., 1966]. The major cause of death (at a median age of 47) is myocardial infarction (MI) [Epstein et al., 1966]. The WS mutation involves a member (*WRN*) of the *RecQ* family of helicases and may perturb DNA replication, repair, recombination, transcription, or chromosomal segregation [Yu et al., 1996]. We now report data on 149 MI cases and age-matched controls suggesting that a polymorphic *WRN* variant is associated with increased risk for MI. Based on our data, homozygosity for a cysteine at amino acid 1367 (the most prevalent genotype) predicts a 2.78 times greater risk of MI (95% confidence intervals: 1.23 to 6.86). The variant was not significantly associated with NIDDM. The two alleles (cysteine vs. arginine) could influence helicase activity, turnover, macromolecular interactions or, alternatively, could be markers for haplotypes influencing *WRN* regulation or reflecting gene action at linked loci. However, given the caveats implicit in genetic association studies, it is imperative that the present results be replicated in independent populations.

KEY WORDS: Werner syndrome, myocardial infarction, diabetes mellitus

Am. J. Med. Genet. 68:494–498, 1997.

INTRODUCTION

The Werner syndrome (WS) is caused by mutations at the Werner helicase locus (*WRN*) [Yu et al., 1996]. Homozygotes or compound heterozygotes [Oshima et al., 1996b] exhibit early onset of various age-related disorders, including

[1] Address reprint requests to T. Miki, Department of Geriatric Medicine, Osaka University Medical School, Suita, Osaka 565, Japan

Contract grant sponsor: Research Project on Health and Aging; Contract grant sponsor: Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists; Contract grant sponsor: National Institute on Aging; Contract grant numbers: AG01571, AG08303.

Received 20 November 1996; Accepted 19 December 1996

Table I. Distribution of *WRN* genotypes among Japanese subjects with a history of myocardial infarction (MI) compared to a group of age-matched Japanese controls and Japanese subjects diagnosed with non-insulin-dependent diabetes mellitus (NIDDM)

		Control	NIDDM	MI
Total number of subjects		198	166	149
	male	185	81	140
	female	13	85	9
Age of subjects (years)				
	mean	53.0	60.3	53.4
	s.d.	±8.36	±10.2	±8.30
	max	72	82	73
	min	30	35	29
Genotype				
	CC	168	140	140
	CR	26	26	9
	RR	4	0	0

the controls (95% CI 1.23 to 6.86, p = 0.009). In contrast, the CC genotype was not associated with NIDDM (odds ratio = 0.9, 95% CI 0.52, 1.78).

DISCUSSION

These results are consistent with, but by no means establish a role for variable expression of the *WRN* helicase in the pathogenesis of atherosclerosis in the general Japanese population and, possibly, in the most populations.

There are several independent lines of evidence that could be interpreted to support such a role. First, it is known that mutation at that locus causes early death attributable to coronary artery atherosclerosis [Epstein et al., 1966]. Second, somatic cells from such homozygous mutant subjects have been shown to be hypermutatable, with forward mutation rates [at the hypoxanthinephosphoribosyltransferase (*HPRT*) locus] that are 10-100 times that of controls, with particularly high rates of deletions [Fukuchi et al., 1989]. Several different types of observations support that conclusion, including cytogenetic studies indicating a propensity of WS cells to undergo chromosomal deletions, reciprocal translocations and inversions ("variegated translocation mosaicism") [Hoehn et al., 1975; Salk et al., 1981], host cell ligation assays of mutation frequency and type in lymphoblastoid cell lines [Rünger et al., 1994], and assays for presumptive *HPRT* mutations carried out with fresh peripheral blood lymphocytes from WS patients [Fukuchi et al., 1990]. Third, somatic cells from WS subjects appear to have an elongated S phase [Takeuchi et al., 1982; Poot et al., 1992]. Fourth, WS somatic cells have sharply limited replicative life spans [Martin et al., 1970]. Fifth, a pioneering study by the late Earl P. Benditt demonstrated that atheromas from black females heterozygous for a G6PD polymorphism are monotypic, consistent with a monoclonal origin and, hence, potentially reflecting somatic mutations [Benditt and Benditt, 1973]. Sixth, helicases may participate in the repair of damaged DNA [Hanawalt, 1994; Tuteja and

atherosclerosis [Epstein et al., 1966]. The major cause of death is myocardial infarction (MI) [Epstein et al., 1966] at a median age of 47. Other forms of severe arteriosclerosis (medial calcinosis and arteriolosclerosis) may also contribute to the age-related pathology [Tollefsbol and Cohen, 1984]. The frequency of reported WS is higher in Japan probably because of the high rate of consanguineous marriage and the awareness of physicians [Goto et al., 1996]. A question of considerable clinical significance is the degree to which not only mutations, but also polymorphisms, may increase the risk of MI in the general population. We now report evidence of such a role in a Japanese population.

MATERIALS AND METHODS

Subject Selection

MI subjects were from cardiovascular specialty hospitals in the Osaka region and were described previously, together with age-matched controls [Zhao et al., 1994; Kamitani et al., 1995]. Control subjects within the desired age group were recruited from Osaka area outpatient clinics for systematic annual check-ups. None had clinical or laboratory evidence of either past myocardial infarctions or any form of diabetes mellitus. Diagnosis of MI utilized coronary angiography, electrocardiography and measurements of heart-specific serum enzymes, as well as clinical criteria. Non-insulin dependent diabetes mellitus (NIDDM) subjects were from outpatient clinics in the Osaka region and were also described previously [Nakagawa et al., 1995]. Diagnosis of NIDDM utilized previously described criteria [National Diabetes Data Group 1979]. All subject groups consisted predominantly of businessmen and housewives. Institutional approval and informed consents were obtained for this study.

Genotyping

Genotype was determined by the presence or absence of the *Pma*CI site in the PCR product amplified from the genomic DNA. The *Pma*CI site is present in an allele coding for arginine, but not in one coding for cysteine. Genomic DNA was amplified with the primer 5'-GCCTAATCAGAATGTTAG TT-3' and antisense primer 5'-TCAGTATTGATGCCTACCTC-3'. PCR product was digested with *Pma*CI, separated on 10% polyacrylamide gel and visualized by ethidium bromide staining. The allele with cysteine yielded a single 193-bp fragment while the allele with arginine yielded 101-bp and 92-bp fragments.

Statistics

The association between genotype and disease was measured by the odds ratio, an estimate of the relative risk appropriate for case-control data [Hennekens and Buring, 1987]. Confidence intervals and hypothesis testing were based on the two-sided Fisher's exact test.

RESULTS

Several polymorphisms in *WRN* were observed during the mutation screening of this locus in WS patients, progeria patients and controls [Oshima et al., 1996a; Oshima et al., 1996b].

As can be seen in Table I, there was a very close match between the ages of control vs. MI subjects, both exhibiting means of about 53 years, with quite comparable standard deviations and ranges. The allele coding for cysteine (C) at amino acid position 1367 predominates in both controls and MI subjects. However, the minor allele coding for arginine (R) at that position is almost three times as prevalent in the control group as in the MI group (Table I). The odds of observing the CC genotype in MI cases was 2.78 times that of

Tuteja, 1996]. However, an alternative interpretation of our results is that the relevant gene action reflects one or more genes in linkage disequilibrium with the C polymorphic variant.

Putative genetic associations are notorious for providing misleading results because of potential biases in the selection of cases and controls. We are unaware of any obvious bias of subject selection in the present study. The Japanese population does not exhibit the striking ethnic and, consequently, genetic heterogeneity observed in the United States and is therefore more favorable for such association studies. However, it will be essential to confirm and extend the present results for independent populations, both Japanese and Caucasian, and to consider *WRN* in quantitative trait locus analysis of MI.

The issue is of great conceptual importance, as it would point to a mechanism of differential susceptibility to atherogenesis based upon the metabolism of nucleic acids. Such a pathogenesis could be independent of or coupled to the widely accepted models of atherosclerosis that are based on gene actions involving the metabolism of lipids [Mahley and Rall, 1995; Goldstein et al., 1995]. Coupling of the two theories could derive from a consideration of the oxidative damage theory of aging [Martin et al., 1996]. Oxidized lipoproteins may activate inflammatory events [Berliner and Heinecke, 1996], which could lead to the generation of active oxygen species, DNA damage, and mutagenesis [Reid et al., 1994]. Differential function of the *WRN* helicase could variably modulate such DNA damage.

ACKNOWLEDGMENTS

We thank the participating subjects. The authors have no commercial interests in the results of these studies. This research was supported by grants from the Research Project on Health and Aging (T.M., J.N.), Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists (Y.L.), and the National Institute on Aging (AG01571 and AG08303) (G.M.M.)

REFERENCES

Benditt EP, Benditt JM (1973): Evidence for a monoclonal origin of human atherosclerotic plaques. Proc Natl Acad Sci USA 70:1753-1756.

Berliner JA, Heinecke JW (1996): The role of oxidized lipoproteins in atherogenesis. Free Radic Biol Med 20:707-727.

Epstein CJ, Martin G, Schultz AL, Motulsky AG (1966): Werner's syndrome: A review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. Medicine 45:177-222.

Fukuchi K, Martin GM, Monnat RJ Jr (1989): Mutator phenotype of Werner syndrome is characterized by extensive deletions. Proc Natl Acad Sci USA 86:5893-5897.

Fukuchi K, Tanaka K, Kumahara Y, Maruno K, Pride MB, Martin GM, Monnet RJ Jr (1990): Increased frequency of 6-thioguanine-resistant peripheral blood lymphocytes in Werner syndrome patient. Hum Genet 84: 249-252.

Goldstein JL, Hobbs HH, Brown MS (1995): Familial hyperchoresterolemia. In Scriver CR, Beaudet AL, Sly WS, Valle D (eds): "The Metabolic and Molecular Basis of Inherited Disease." New York: McGraw-Hill, pp. 1981-2130.

Goto M, Miller RW, Ishikawa Y, Sugano H (1996): Excess of rare cancers in Werner syndrome (adult progeria). Cancer Epidemiol Biomarkers Prev 5: 239-246.

Hanawalt PC (1994): Transcription-coupled repair and human disease. Science 266:1957-1958.

Hennekens CH, Buring JE (1987): "Epidemiology in Medicine." Boston: Little, Brown & Company.

Hoehn H, Bryant EM, Au K, Norwood TH, Boman H, Martin GM (1975): Variegated translocation mosaicism in human skin fibroblast cultures. Cytogenet Cell Genet 15:282-295.

Kamitani A, Rakugi H, Higaki J , Ohishi M, Shi SJ, Takami S, Nakata Y, Higashino Y, Fujii K, Mikami H, Miki T, Ogihara T (1995): Enhanced predictability of myocardial infarction in Japanese by combined genotype analysis. Hypertension 25:950-953.

Mahley RW, Rall SC Jr (1995): Type III hyperlipoproteinemia (dysbeta-lipoproteinemia): The role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In Scriver CR, Beaudet AL, Sly WS, Valle D (eds): "The Metabolic and Molecular Basis of Inherited Disease." New York: McGraw-Hill, pp. 1953-1980.

Martin GM, Austad SN, Johnson TE (1996): Genetic analysis of aging: Role of oxidative damage and environmental stresses. Nature Genetics 13:25-34.

Martin GM, Sprague CA, Epstein CJ (1970): Replicative life-span of cultivated human cells. Effects of donor's age, tissue, and genotype. Lab Invest 23:86-92.

Nakagawa Y, Ikegami H, Yamato E, Takekawa K, Fujisawa T, Hamada Y, Ueda H, Uchigata Y, Miki T, Kumahara Y, Ogihara T (1995): A new mitochondrial DNA mutation associated with non-insulin-dependent diabetes mellitus. Biochem Biophys Res Commun 209:664-668.

National Diabetes Data Group (1979): Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039-1057.

Oshima J, Brown WT, Martin GM (1996a): No detectable mutations at Werner helicase locus in progeria. Lancet 348:1106.

Oshima J, Yu C-E, Piussan C, Klein G, Jakowski J, Balci S, Miki T, Nakura J, Ogihara T, Ellis J, Smith MAC, Melagrano MI, Fraccaro M, Scappaticci S, Matthews J, Ouais S, Jarzebowicz A, Schellenberg GD, Martin GM (1996b): Homozygous and compound heterozygous mutations at the Werner syndrome locus. Hum Molec Genet in press.

Poot M, Hoehn H, Rünger TM, Martin GM (1992): Impaired S-phase transit of Werner syndrome cells expressed in lymphoblastoid cell lines. Exp Cell Res 202:267-273.

Reid TM, Feig DI, Loeb LA (1994): Mutagenesis by metal-induced oxygen radicals. Environ Health Perspect [Suppl]102:57-61.

Rünger TM, Bauer C, Dekant B, Moller K, Sobotta P, Czerny C, Poot M, Martin GM (1994): Hypermutable ligation of plasmid DNA ends in cells from patients with Werner syndrome. J Invest Dermatol 102:45-48.

Salk D, Au K, Martin GM (1981): Cytogenetics of Werner's syndrome cultured skin fibroblasts: Variegated translocation mosaicism. Cytogenet Cell Genet 30:92-107.

Takeuchi F, Hanaoka F, Goto M, Yamada M, Miyamoto T (1982): Prolongation of S phase and whole cell cycle in Werner's syndrome fibroblasts. Exp Gerontol 17:473-480.

Tollefsbol TO, Cohen HJ (1984): Werner's syndrome: An under-diagnosed disorder resembling premature aging. Age 7:75-88.

Tuteja N, Tuteja R (1996): DNA helicases: The long unwinding road. Nature Genet 13:11-12.

Yu C-E, Oshima J, Fu Y-H, Hisama F, Wijsman EM, Alsch R, Matthews S, Nakura J, Miki T, Ouais S, Martin GM, Mulligan J, Schellenberg GD (1996): Positional cloning of the Werner's syndrome gene. Science 272:193-194.

Zhao Y, Higashimori K, Higaki J, Kamitani A, Ooichi M, Katsuya T, Miki T, Mikami H, Kumahara Y, Minamino T, Ogihara T (1994): Significance of the deletion of polymorphism of the angiotensin converting enzyme gene as a risk factor for myocardial infarction in Japanese. Hypertension Res 17:55-57.